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Brevet canadien / Canadian Patent

Le commissaire aux brevets a reçu une demande de délivrance de brevet visant une invention. Ladite requête satisfait aux exigences de la *Loi sur les brevets*. Le titre et la description de l'invention figurent dans le mémoire descriptif, dont une copie fait partie intégrante du présent document.

Le présent brevet confère à son titulaire et à ses représentants légaux, pour une période expirant vingt ans à compter de la date du dépôt de la demande au Canada, le droit, la faculté et le privilège exclusif de fabriquer, construire, exploiter et vendre à d'autres, pour qu'ils l'exploitent, l'objet de l'invention, sauf jugement en l'espèce rendu par un tribunal compétent, et sous réserve du paiement des taxes périodiques.

The Commissioner of Patents has received a petition for the grant of a patent for an invention. The requirements of the *Patent Act* have been complied with. The title and a description of the invention are contained in the specification, a copy of which forms an integral part of this document.

The present patent grants to its owner and to the legal representatives of its owner, for a term which expires twenty years from the filing date of the application in Canada, the exclusive right, privilege and liberty of making, constructing and using the invention and selling it to others to be used, subject to adjudication before any court of competent jurisdiction, and subject to the payment of maintenance fees.

BREVET CANADIEN **2,020,633** CANADIAN PATENT

Date à laquelle le brevet a été accordé et délivré	**1997/08/19**	Date on which the patent was granted and issued
Date du dépôt de la demande	**1990/07/06**	Filing date of the application
Date à laquelle la demande est devenue accessible au public pour consultation	**1991/01/18**	Date on which the application was made available for public inspection

Commissaire aux brevets / Commissioner of Patents

Industrie Canada Industry Canada

3258 (12-96-01)

Canada

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Canadian Intellectual Property Office

(12)(19)(CA) **Brevet-Patent**

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(54) **MUTANT DE PSEUDOMONAS, SOUCHE YZH, PROCEDE DE PRODUCTION D'UNE SOLUTION NUTRITIVE 851 YZH EN UTILISANT LADITE SOUCHE**

(54) **MUTANT OF PSEUDOMONAS, A STRAIN YZH, AND A PROCESS FOR PRODUCING 851 YZH NUTRIENT SOLUTION BY APPLICATION OF THE STRAIN**

(57) L'invention concerne une souche mutante d'une bactérie du genre Pseudomonas appelée souche YZH et l'utilisation de cette souche au moyen d'un procédé de fermentation pour produire une solution nutritive de protéines d'organisme unicellulaire à partir de protéines végétales. La solution nutritive ainsi obtenue convient à l'alimentation humaine et animale et possède des vertus bienfaitrices agissant chez les humains et les animaux : elle réduit le saignement de la muqueuse gastrique, stimule et améliore la production l'hématopoïese; inhibe la croissance des cellules cancéreuses et retransforme les cellules cancéreuses en cellules normales. Les principales caractéristiques de la souche YZH sont les suivantes : bâtonnet de coloration jaune pâle, possédant au moins une flagelle, capable de réduire les nitrates et de liquéfier la gélatine, incapable de produire des pigments hydrosolubles ni de former du poly-béta-hydroxybutyrate et, en particulier, la souche possède dans son ADN un fragment gamma décelable au moyen d'une sonde d'ADN spécifique du fragment gamma.

(57) This invention relates to a mutant pseudomonas identified as strain YZH and the use of the strain by means of a fermentation process to produce a single cell protein nutrient solution from plant protein. The resulting nutrient solution is suitable as food for animals and human, and has salutory effects on animals and human such as reducing bleeding of the gastric mucosa, stimulating and improving hemogenesis and inhibiting cancer cell growth and traversing the cancer cell into its normal cell. The main characteristics of the strain YZH are that it is a rod in light yellow color, has at lease one flagellum, has the ability to reduce nitrate and to liquidize gelatine, is not able to produce water soluble pigment, and to form poly-.beta.-hydroxy butyrate and in particular, has a .gamma. fragmentin its DNA which is detectable by means of a specific .gamma. fragment DNA probe.

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A MUTANT OF PSEUDOMONAS, A STRAIN YZH, AND A PROCESS FOR PRODUCING 851 YZH NUTRIENT SOLUTION BY APPLICATION OF THE STRAIN

FIELD OF THE INVENTION

The present invention relates to the production of single cell protein from plant protein by fermentation or incubation, using large scale industrial type equipment. The single cell protein is produced by the action of a mutant strain of pseudomonas on plant protein. The resulting product is a nutrient liquid or solution, or solid product made therefrom which is edible by humans and animals.

BACKGROUND OF THE INVENTION

Fermentation is an ancient and widely practised art used to produce food and drink such as, sourdough bread, sauerkraut from cabbage, wine and beer. In some regions of the world where arable land is being increasingly used for non-food purposes, other food producing resources are looked for by means of the fermentation method. In the past, many microorganisms have been studied and attempts have been made to adapt them to industrial fermentation methods in order to produce proteins, in particular, single cell protein, which can be easily absorbed by the human body.

United States Patent No. 4,877,739 discloses a group of autogenic antiammonia azotobacter designed as 851 yellow that is mutated from Azotobacter vinelandii, having the capability of antiammonia nitrofixation. This microorganism can also be used for manufacturing a single cell protein liquid enriched in Se, Zn, vitamin E (Ve), vitamin B (Vb), vitamin K (Vk), anticancer and antiaging tonic medicines. It also can be used

for making bacterial fertilizer, eel and animal forage, additive, and antiseptic and binder.

Our invention discloses a process for manufacturing single cell protein from plant protein with a mutant strain of pseudomonas, or natural mutant or induced mutant of the said mutant strain. The resulting fermentation product, either a nutrient solution, a solid nutrient product or a product derived therefrom, is edible by humans and animals. The nutrient solution of the present invention has the effects of antiaging, stimulating and improving hemogenesis in animals, protecting gastric mucosa of animals and humans, especially, inhibiting cancer cell growth in vitro and in vivo in animals and humans, even reverting the cancer cells to their normal status.

SUMMARY OF THE INVENTION

This invention provides a method for producing a single cell protein nutrient solution rich in more than twenty amino acids as required by the human body and various trace elements by means of a mutant of pseudomonas, or natural mutant and induced mutant thereof, in the conventional industrial fermentation method. The strain of the microorganism or pseudomonas, designated as strain YZH, is particularly useful in manufacturing single cell protein from a soybean material, although it can also be used with other plant material such as starch, potatoes and sweet potatoes. Soybeans or soybean protein which has been incubated or fermented with strain YZH according to the present invention is effectively converted into a single cell protein which is easily assimilated by the human body. Typically, this fermentation is carried out in aqueous medium to produce a single cell protein nutrient

solution herein designated as 851 YZH nutrient solution or 851 YZH NS. As used herein, solution, liquid or nutrient solution means a true solution, or a suspension or mixture of a solid in a liquid to give a resulting solution - like fluid mass.

The microorganism of this invention is a mutant of pseudomonas designated as strain YZH, said pseudomonas having all the identifying characteristics of the sample on deposit with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852 and assigned A.T.C.C. Deposit No. 53980.

This invention also relates to using the pseudomonas, strain YZH, in a conventional industrial fermentation process to produce a single cell protein nutrient solution comprising using pseudomonas YZH, or cultivated pseudomonas YZH as the single cell protein producing strain in a producing medium which may be a soybean, soybean derived protein or starch medium, or other media susceptible to the manufacture of single cell proteins. Additionally, the invention is also related to effects of the nutrient solution on mammals, including humans.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram of the cellular growth of Human Gastric Adenocarcinoma Cell, MGC80-3 cell in vitro by the effect of 851 YZH nutrient solution, wherein the percentage is a concentration of the solution.

Fig. 2 is a diagram of the cellular mitotic index of the MGC80-3 cells in vitro by the effect of 851 YZH nutrient solution.

Fig. 3 is a view of the MGC80-3 cells under a phase contrast microscope.

Fig. 4 is a view of the MGC80-3 cells which are treated with 15% 851 YZH solution, stained with hematoxylin-eosin (H.E.) under a phase contrast microscope.

Fig. 5 is a scanning electron micrograph of the MGC80-3 cells.

Fig. 6 is a scanning electron micrograph of the MGC80-3 cells which are treated with 20% 851 YZH nutrient solution.

Fig. 7 is a transmission electron micrograph of the MGC80-3 cell, wherein:

N:	nucleus	Nu:	nucleclus
G:	Gogli complex	M:	mitochondria
ER:	endoplasmic reticulum		

Fig. 8 is a transmission electron micrograph of the MGC80-3 cells which are treated with 20% YZH nutrient solution.

DETAILED DESCRIPTION OF THE INVENTION

The microcrganism of this invention is a mutant pseudomonas designated Pseudomonas YZH and having all the characteristics of the sample on deposit with the American Type Culture Collection and assigned A.T.C.C. Deposit No. 53980. The strain YZH is found in and separated from soybeans or soybean derived material. The characteristics of the strain YZH are that it is a straight rod light yellow in color, it has at least one flagellum, the thallus is of 0.5-0.7 x 1.2-2.0 um in size, it is a chemosynthetic heterotroph, it is unable to produce water-soluble pigment, it does not have an ability to form intracellular poly-β-hydroxy butyrate, and to produce arginine dihydrolase, it does not have an action of denitrification, it has the ability to reduce nitrates and to liquidize gelatine, it is obligately aerobic,

the growth temperature is about 10-41°C, the preferable PH value is 4.5-9.0, the content of guanine and cytosine in DNA of the strain is 65.4-67.7 mol%, and the DNA of the strain contains a Y fragment which can be detected by a special Y fragment DNA probe. All the features as mentioned above are the differential features of the YZH strain compared to other gena of pseudomonas. When strain YZH is used in a nitrogen containing medium as taught in this invention, it can produce a single cell protein solution enriched in more than twenty amino acids and a variety of trace elements and vitamins. Analysis of 100 ml samples of the nutrient solution produced by YZH is found to contain the following range of substances: 350-800 mg of amino acids, 0.2-0.5 mg of vitamin E, 0.05-0.1 mg vitamin B_2, 0.7-1.0 mg nicotinic acid (vitamin PP) or Niacin, 0.04-0.08 ppm Se, 0.2-0.5 ppm Zn, 0.08-0.24 ppm Mo, 0.08-0.24 ppm Co, 0.05-0.06 ppm Mn, 0.07-0.2 ppm Cu and 0.1-0.7 ppm Fe; 1.21-1.38 g dry substance, 1.21-2.2 g proteins, 0.11-0.13 g lipids, and other substances.

The process for producing 851 YZH NS through the use of the YZH pseudomonas includes the following steps:

A culture medium for cultivating YZH is placed in a conventional fermentation tank which is equipped with a mixing means and gas inlet/outlet means so that the tank content can be homogenized and also aerated by the introduction of filtered aseptic air needed by YZH and other attendant microorganisms during the period of culturing. The culture medium is soybeans or starch or other suitable materials as provided herein. It is seeded in a two-step process using YZH seed culture as a seed culture for 38-54 hours. After the seeding is completed, the resulting thallus is incculated into a pre-autoclaved production medium of soybeans, starch or

other suitable material for a third continuous culture or incubation. The production medium can contain other materials such as nutritionally important trace elements in addition to soybeans or starch as is described before. Once the thallus has been inoculated for about 24-72 hours with filtered aseptic air passing through the mass, incubation conditions are: aeration rate of 1:0.6 - 1.2(mass/air) v/v min; mixing speed of 180-260 rpm; and the temperature of 28-38°C. After the incubation is finished, the resulting cultured broth is autoclaved and harvested. The broth (nutrient solution, or liquid) can be packaged directly to give a product suitable for human use. Alternative products can also be obtained to fulfill a variety of needs by means of other processing. For example, the culture solution can be centrifuged and precipitated and then filtered by a thin membrane or super filtration to give a product which is edible directly by humans and animals.

Furthermore, the filtered solution may be further extracted with an organic solvent such as alcohol, acetone, or ethyl acetate, or extracted with column extractor such as an ion exchange column, or wide-aperture resin column to give a final product, which contains a concentrated nutrition component. The culture solution can optionally be added in any convenient way into foods or drugs to produce a product containing 851 YZH pseudomonas. The solution can also be dried to give a solid product. The solution can be use as a replacement for water and eggs in the baking of bread, cakes and the like, or in other foods. The culture solution can also be used in cosmetic preparation.

In the method described above, the following production media have been used. Components are listed by weight with

water making up the remainder. Proper amounts of trace elements necessary to the human body in addition to these media are added. The benzoic acid or sodium benzoate can also be used in said media. The amounts of such elements are typically in the range of 1.5-100ppm.

1. Soybean medium

Soybean	5-10%
or Soybean milk or bean cake (by weight of soybean)	5-15%
Yeast extract	0.02-0.5%
or yeast powder	0.02-0.5%
or peptone, beef extract	0.02-0.3%
$CaCl_3$	0.05-0.25%
KH_2Po_4	0.02-0.1%
$MgSo_4$	0.01-0.05%
NaCl	0.01-0.04%
Na_2MoO_4	5.0-30ppm
Na_2SeC_3	2.5-15ppm
$ZnSo_4$	2.5-40ppm
$CoCl_2$	5.0-20ppm

2. 1640 medium

The medium 1640 which is well known can also be used for the culture of YZH pseudomonas.

The 851 YZH nutrient solution, used as is, or in any of its forms, possesses salutary effects including promoting or protecting the health of the animals, especially inhibiting the growth of cancer cells in vivo and in vitro in animals and humans. In order to more fully demonstrate the salutary effects of 851 YZH NS and its derivative, the following experiments and their results are presented.

Experiment 1: Anti-lipid Peroxidative Effect

The oxygen free radical, hydroxy free radical and peroxide, especially lipid peroxide in the human and animal body can kill cells, and are believed to cause acute and chronic diseases and senility of humans and animals. Some foods or drugs having an effect of direct or indirect anti-lipid peroxide may be able to prevent cancers or tumors and senility of humans and animals who do not have enough anti-peroxide activity or have a hyperactivity of lipid peroxide. (Harman, D Free Radicals in Mol, Biol, Aging & Diseases 1984, Raven Press, New York, pp1-12).

Kunming mice weighing 17.8 ± 1.75g were used and randomly divided into several groups (A,B,C) of 10 mice each according to body weight. The control group was designated as group (A). Mice from three groups were given the same diet. In addition, mice of group (C) were given 851 YZH nutrient solution for 13 consecutive days, whereas groups (A) and (B) were given tap water. All mice were fasted on the 14th day. After fasting, mice of group (A) were administered paraffin oil (a solvent of CCl_4) on the 14th day, intra peritoneally at about 4 p.m. The mice of group (B) and group (C) were administered with CCl_4 intra peritoneally on the 14th day at 4 p.m. The amount of administration was 1.87m mol/kg.

On the 15th day, the animals were sacrificed and liver samples were prepared. Malonaldehyde (MDA) in the liver was analyzed according to the method of Hiroshi Ohkawa et al. (Anal. Biochem. 95:351-356 (1979)), 1,1,3,3- tetramethoxy-propane (Japan TCI, E,P,Grade) was used as the standard for the analysis of lipid peroxide. MDA was spectrophotometrically determined in n mol/g and the data were statistically analyzed (M±SD). The results were given below:

TABLE 1

Group	No. of Mice	MDA n mol/g liver	P value Compared to control group (A)	P value Compared to CC14 group (B)
Control (A)	10	119.5 ± 27.2	-	-
CCl_4 (B)	10	1698.9 ± 287.6	<0.001	-
CCl_4 851 YZH(C)	9	1350.2 ± 170.8	<0.001	<0.01

The results indicate that 851 YZH NS possesses potent anti-liquid peroxide activity by substantially lowering the MDA level. This property might be useful in combatting aging.

Experiment 2: Effect of 851 YZH NS on the hemoglobin and leucocyte total and classification of WBC of new-born mice

Twelve pregnant Kunming mice were fed normal diet and tap water. After parturition, the twelve mice, with their respective newborn, were randomly divided into control group (A) and an 851 YZH NS test group (B). The above noted mice of control group (A) were maintained on normal diet and tap water, mice of group (B), however, had their diet supplemented with 851 YZH NS during the suckling period and after weaning. The test duration ran for 15 days after weaning. At the end of the 15 day period, blood samples of the newborn mice were taken from the eyeballs of the newborn mice for analysis. The results were as follows:

TABLE 2

Effect of 851 YZH NS on leucocyte total and hemoglobin

Group	No. Newborns	Hemoglobin (g.x±SD)	P*	Total WBC (X10/mm X±SD)	P**
Control (A)	34	10.0±1.2	-	5.373±1.040	-
851 YZH (B)	33	10.7±1.0	<0.01	6.279±1.910	<0.05

* Compared with the control group.

** Both tested, compared with the control group.

TABLE 3

Effect of 851 YZH NS on classification of WBC

Group	No. Newborns	Nutrophil (% x±SD)	P1*	Lymphocyte (% X±SD)	P2*
Control (A)	34	58±10	-	42±9	-
851 YZH NS(B)	33	41±11	<0.001	59±11	<0.001

* Compared with the control group, both tested.

The above data indicates that the feeding of 851 YZH NS, which contains more than 20 amino acid, several different vitamins and various trace elements, results in an increase in lymphocytes in the newborn mice which can be taken as a stimulation and improvement of a mouse's ability to produce these cells.

Experiment 3: Therapeutic effect of 851 YZH NS on acute injury to the gastric mucosa of rats

Sixteen male and female Wistar rats, weighing 220±20g, were used in this experiment. These rats were randomly divided into control group (A) and 851 YZH NS (B). Group (A) was fed a normal diet and given tap water. Group (B) had a diet containing 851 YZH NS, each of them had about 8ml of the solution a day. After being fed for 15 days, two groups of rats had nothing to eat for 14 hours. Each rat was given 2.5mg indomethacin, once daily, for two days. Two days later, the rats were sacrificed and their stomachs removed and washed and observed under the naked eye.

Six rats in group (A), 2 rats in group (B) had acute bleeding in the gastric mucosa, but bleeding of mice in group (A) were significantly more severe. The bleeding in gastric mucosa of 6 group (A) rats and 2 group (B) rats were locally restricted as to being spotted, stripped or stretched. Diffuse haemorrhaging of the gastric mucosa occurred in 1 group (A) rat. However, whereas the group (A) showed 6-12 spot haemorrhages, the 851 YZH NS group showed only 2-5 spot haemorrhages. These results indicate that the administration of 851 YZH NS can significantly reduce gastric mucosal bleeding.

Experiment 4: Effect of 851 YZH NS on the human chronic gastritis

Twenty patients with chronic gastritis were diagnosed through pathological examinations. They were 21-62 years old, 10 male and 10 female, and they all had obvious clinical symptoms.

All the patients were randomly divided into two groups, control group and test group, each group consisted of 10 patients. The control group was administered with vitamin C for 21 days. The test group was administered orally with 851 YZH NS, three times a day, 80ml once, for 3 weeks. The results were given below:

TABLE 4

Symptoms	Control Group (n=10)			851 YZH NS Group (n=10)		
	Cure	Relief	Ratio	Cure	Relief	Ratio
abdominal discomfort	1/3	2/8	38	4/9	3/9	78
abdominal distension	-	2/10	20	6/7	-	86
acid regurgitation	-	1/7	14	2/4	-	50
belching	1/9	2/9	33	3/8	2/8	63
nausea & vomiting	1/4	-	25	4/5	-	80
anorexia	1/7	2/7	43	4/5	1/5	100

As noted above, compared with the control group, 851 YZH NS can improve the clinical symptoms of stomach disorder and relieve chronic gastritis.

Experiment 5: Effect of 851 YZH NS on cancer cell growth

851 YZH NS of our invention possessed a strong inhibition of carcinoma cell growth, for example human gastric adenocarcinoma cells, MGC80-3 cells. Said cells were treated individually with 10%, 15% and 20% 851 YZH NS to give a good result. (See Fig. 1 and Fig. 2).

Experiment 6: Effect of 851 YZH NS on human cancer cell observed under the electron microscope

Human gastric adenocarcinoma, MGC80-3 cells, treated with 15% 851 YZH NS showed that there was an evident morphological change in the cells stained with H.E. under phase contrast microscope (see Fig. 2 and Fig. 3). In Fig. 4 and Fig. 5 scanning electron micrograph of the MGC80-3 cells showed that there were abundant filopodia existed at MGC 80-3 cell's margin and microvilli densely covered their cells surface. But after the treatment of 20% 851 YZH NS, the microvilli at the cell surfaces disappeared. Said cells had long cytoplasmic projections and lamellipodia at the cell margins, and emerged with many wrinkle-like structures and bubbles.

In Fig. 6 and Fig. 7, transmission electron micrograph showed that the MGC80-3 cell was characterized with high nuclear-cytoplasmic ratio, irregular nuclear shape, large nucleolus, increasing heterochromatin and non-developmental organelles in cytoplasm, etc. After treatment with 20% 851 YZH NS for about 7-9 days, the MGC80-3 cells had produced a series of ultrastructural changes similar to those of their

corresponding normal cell, such as the nucleus (N) was regular, nucleolus (Nu) lessened, heterochromatin reduced and euchromatin increased. Golgi complex (G) and mitochondria (M) were developed and rough endoplasmic reticulum (RER) increased as indicated in Fig. 7. The above results indicate that 851 YZH NS has the effect of inhibiting or killing cancer cells in vitro, even on reversing the cancer cells into their normal condition. The inhibition and killing action on the cancer cells will take place at 12-24 hours in the treatment of said nutrient solution. Those cells that are not killed are cultured in the nutrient solution continuously for about 7-9 days. They can gradually change into their corresponding normal cells.

Experiment: 7 The result of mice heterotransplantation with the MGC80-3 cells and the cells treated with 15% 851 YZH NS

Thirty Kunming mice, weighing 17.5 ± 1.65g, were randomly divided into three groups (A,B,C), each group having 10 mice.

All the mice of groups (A), (B) and C were given the MGC80-3 cells at the left forelegs. After feeding for 14 days, the mice were sacrificed. It was found that there were tumoriferous cases at the left foreleg armpits of the mice of all groups.

But, the mice of groups (A) and (C) were administered with the MGC80-3 cells that were treated with 15% NS for 3 days at the right forelegs. After the mice (20) were fed for 3 days, 10 mice had small tubercles at their right foreleg armpits.

In addition, the mice of group (B) were given the MGC80-3 cells which were treated with 15% NS for 10 days, at their right forelegs. None of the mice had tumors.

The results indicate that 851 YZH NS has a strong inhibiting effect on cancer or tumor growth in animal body.

Example 1

A 5 ton fermentation tank, equipped with mixing and aeration means was charged to 40% capacity. The fermentation medium was a 10% soybean medium consisting of, by weight, 200kg soybean that is processed by selecting, washing, milling and removing the residue, 800g yeast extract, 1000g KH_2PO_4, 100g $MgSO_4$, 5kg $CACO_3$, 400g NaCl, 20g Na_2MOO_4, 5g Na_2SeO_3, 20g $ZaSO_4$, 20g $CoCl_2$, 1kg benzoic acid and water. The inoculum was 10% and consisted of a YZH pseudomonas cultured for 48 hours in a one-tenth weight of above mentioned medium in a 0.5 ton seed fermentation tank, which was charged with 200 kilograms of the fermentation medium mentioned above. The YZH pseudomonas was added in two stages as a first, second seed culture for about 48 hours. The inoculum was cultured at a temperature of 30°C, stirring speed of 260 rpm and aeration rate of 1:0.8 v/v min (mass/air). After the seed culture was complete, the inoculum was added in the 5 ton fermentation tank for a third continuous inoculation. The fermentation medium and inoculum were allowed to react in the fermentation tank for a period of about 48 hours. The resulting broth (nutrient solution) was then autoclaved at 90°C and atmospheric pressure for a half hour, bottled on an assembly line and autoclaved again at an elevated temperature for a half hour to give the final product. Said final product, a solution, can be dried to give a solid product. Analysis of 100 ml of the broth showed it contained 2.2% dry material, 1.38% proteins, 0.13% lipids, 0.09% carbohydrates, and vitamins, and minerals as is shown in Table 5.

TABLE 5

Analytical Report of 851 YZH NS

Constituents	Unit	Amount	Constituents	Unit	Amount
edible part	g	100			
water	g	97.8	aspartate	mg	66.5
protein	g	1.38	glutamate	mg	78.8
lipids	g	0.13	serine	mg	34.9
carbohydrate	g	0.09	proline	mg	28.4
heat capacity	Kcal	7.1	glycine	mg	34.9
edible fiber	g	-	alanine	mg	41.2
ash	g	0.60	threonine	mg	31.5
----------			valine	mg	34.8
K	mg	59.5	methionine	mg	15.0
Na	mg	7.7	isoleucine	mg	24.0
Ca	mg	4.9	leucine	mg	30.9
Fe	mg	0.6	tyrosine	mg	37.2
Zn	mg	0.3	phenylalanine	mg	28.5
Cu	mg	0.07	lysine	mg	18.5
Mn	mg	0.05	tryptophan	mg	15.0
Mg	mg	4.8	histidine	mg	17.1
P	mg	27.7	arginine	mg	42.0
Se	mg	0.04	----------		
----------			myristic acid	%	7.1
vitamin E	mg	0.22	palmitic acid	%	14.3
vitamin B2	mg	0.07	stearic acid	%	75.5
nicotinic acid	mg	0.81	oleic acid	%	0.9

Example 2

The tank used in example 1 was used. The fermentation medium was at 15% soybean milk medium (by weight of soybean) consisting of 850g yeast extract, 1100g KH_2PO_4, 250g $MgSO_4$, 4g$CaCO_3$, 430g Nacl, 30gNa_2MoO_4, 5g Na_2SeO_3, 15g $ZnSO_4$, 15 $CoCl_2$, 1 kg sodium benzoate and water. The inoculum consisted of YZH pseudomonas (1%) added in two stages as a first and second seed culture for about 50 hours. The inoculum was cultured at a temperature of 27°C stirring speed of 200 rmp and aeration rate of 1:1 v/v min (mass/air). After the seed culture was finished, the inoculum was added in the 5 ton tank for a third continuous incubation. The fermentation medium and inoculum were allowed to react in the fermentation tank for a period of about 65 hours. The resulting cultured broth was autoclaved and harvested at 110°C and barometric pressure for about one hour and autoclaved again at 120°C, and conventional pressure for 2 hours to give a final product, that is our nutrient solution.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A mutant strain of pseudomonas identified as YZH and capable of converting plant protein to single cell protein, said strain being a straight rod light yellow in colour, having at least one flagellum, a chemosynthetic heterotroph, obligatorily aerobic, not able to produce water soluble pigment, and to form intracellular polyhydroxy-butyrate, and to produce arginine dihydrolase, and to have no action of denitrification, and able to reduce nitrates and to liquidize gelatine, the growth temperature being about 10-41°C, the content of guanine and cytosine in DNA of the strain being 65.4 - 67.7 mol%, and the DNA of the strain containing a Y fragment and said strain having identifying characteristics of the sample on deposit with the American Type Culture Collection, 12301 Parklaw Drive, Rockville, Maryland, 20852, and assigned A.T.C.C. Deposit No. 53980.

2. A mutant strain as in claim 1 wherein the growth pH is 4.5 to 9.

3. The mutant strain of pseudomonas in accordance with claim 1 or claim 2, wherein said Y fragment in the DNA of the strain is detected by a special Y fragment DNA probe.

4. A process for producing a single cell protein nutrient solution by using the mutant strain of pseudomonas as claimed in claim 1 or claim 2 as a solution production strain and plant protein containing medium being used in a fermentation procedure to produce said nutrient solution.

5. The process in accordance with claim 4, wherein said plant protein containing medium is an aqueous medium comprising (by weight):

Soybean medium	
Soybean	5-10%
or Soybean milk or bean cake (by weight of soybean)	5-15%
Yeast extract	0.02-0.5%
or yeast powder	0.02-0.5%
or peptone, beef extract	0.02-0.3%
$CaCl_3$	0.05-0.25%
KH_2Po_4	0.02-0.1%
$MgSo_4$	0.01-0.05%
NaCl	0.01-0.04%
Na_2MoC_4	5.0-30ppm
Na_2SeO_3	2.5-15ppm
$ZnSo_4$	2.5-40ppm
$CoCl_2$	5.0-20ppm

6. A nutrient solution comprising an aqueous solution of single cell protein and trace elements, said nutrient solution being obtainable by fermentation of a plant protein containing medium described in claim 5, with a mutant strain of pseudomonas identified as YZH and capable of converting plant protein to single cell protein, said strain being a straight rod light yellow in colour, having at least one flagellum, a chemosynthetic heterotroph, obligatorily aerobic, not able to produce water soluble pigment, and to form intracellular polyhydroxy-butyrate, and to produce arginine dihydrolase, and to have no action of dentrification, and able to reduce nitrates and to liquidize gelatine, the growth temperature

being about 10-41°C, the growth pH being 4.5 - 9.0, the content of guanine and cytosine in DNA of the strain being 65.4 - 67.7 mol%, and the DNA of the strain containing a Y fragment and said strain having identifying characteristics of the sample on deposit with the American Type Culture Collection, 12301 Parklaw Drive, Rockville, Maryland, 20852, and assigned A.T.C.C. Deposit No. 53980.

7. The nutrient solution in accordance with claim 6 for use as a medicament having an anti-aging effect in mammals.

8. The nutrient solution in accordance with claim 6, for use as a medicament having the effect of stimulating and improving hemogenesis in mammals.

9. The nutrient solution in accordance with claim 6, for use as a medicament having a therapeutic effect on stomatic disorder in mammals and humans.

10. The nutrient solution in accordance with claim 6, for use as a medicament having an effect of inhibiting cancer cells growth and reversing the cancer cells into normal cells in mammals and humans.

11. The nutrient solution in accordance with claim 6, for use as a medicament having a therapeutic effect on acute bleeding of the gastric mucosa in mammals.

12. A product containing the nutrient solution as claimed in claim 6 as an additive or a replacement in human or animal foods or drugs.

13. A product containing the mutant strain of pseudomonas as claimed in claim 1 or 2 as an additive or a replacement in human or animal foods or drugs.

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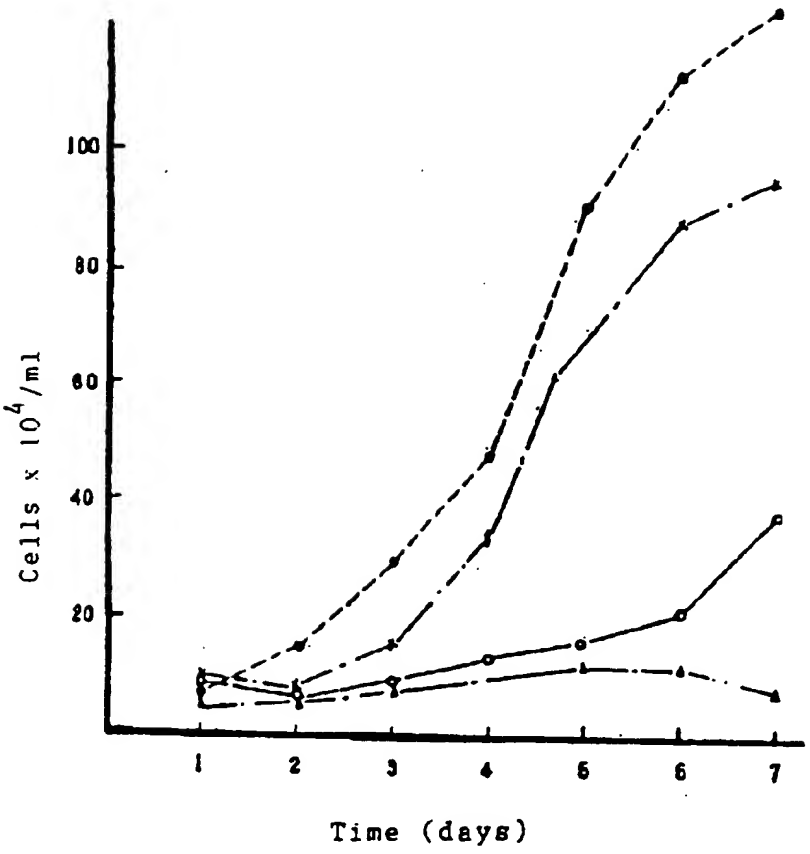

Fig. 1

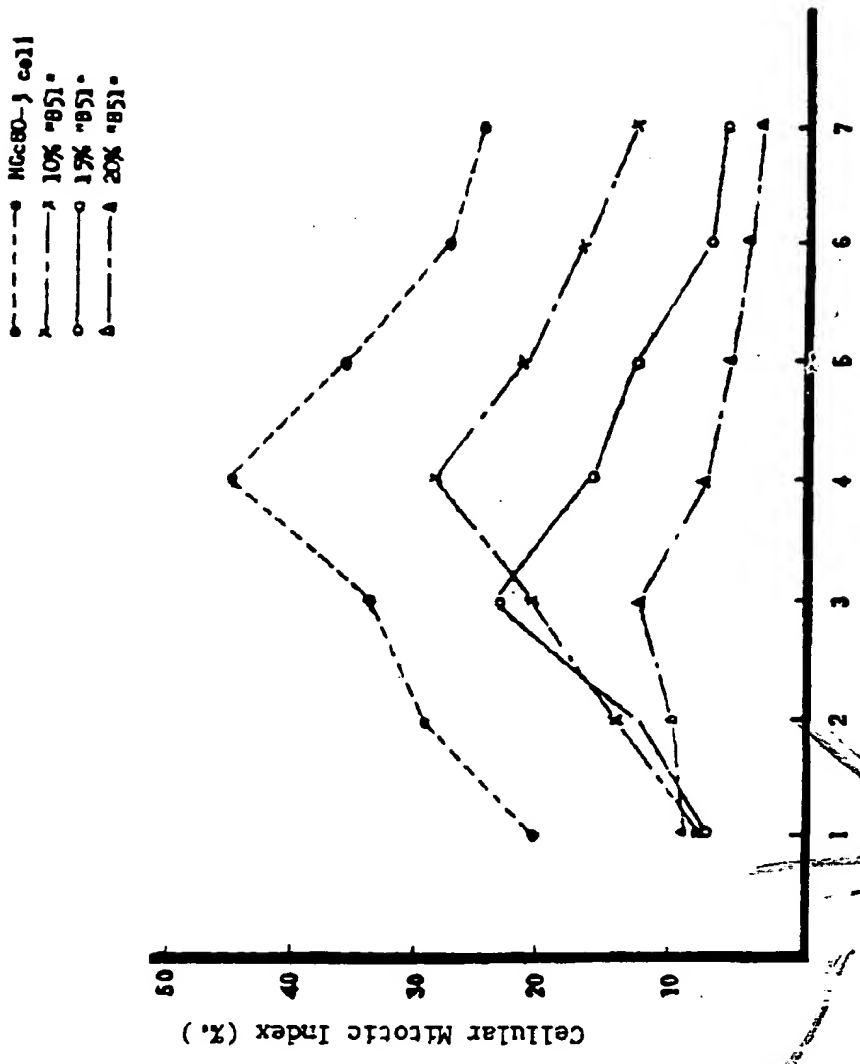
MCeBD-1 cell
10% "851"
15% "851"
20% "851"
Cellular Mitotic Index (%)
50
40
30
20
10
1
2
3
4
5
6
7
Time (Days)

Fig. 2

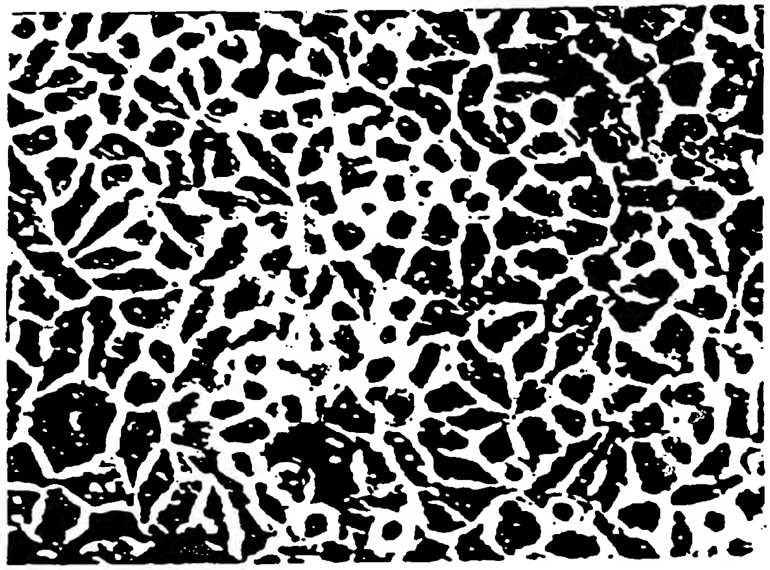

Fig. 3

Fig. 4

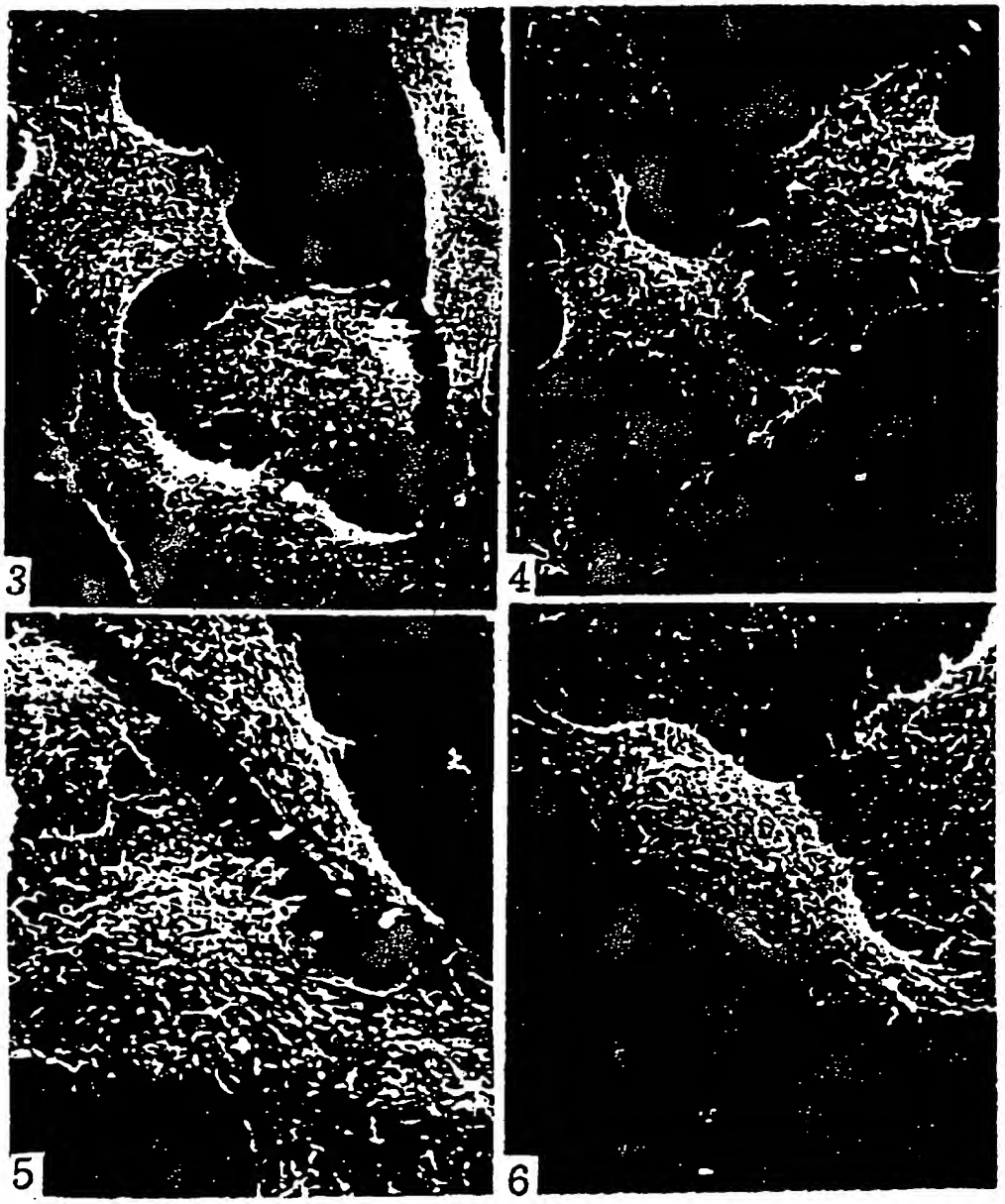

Fig. 5

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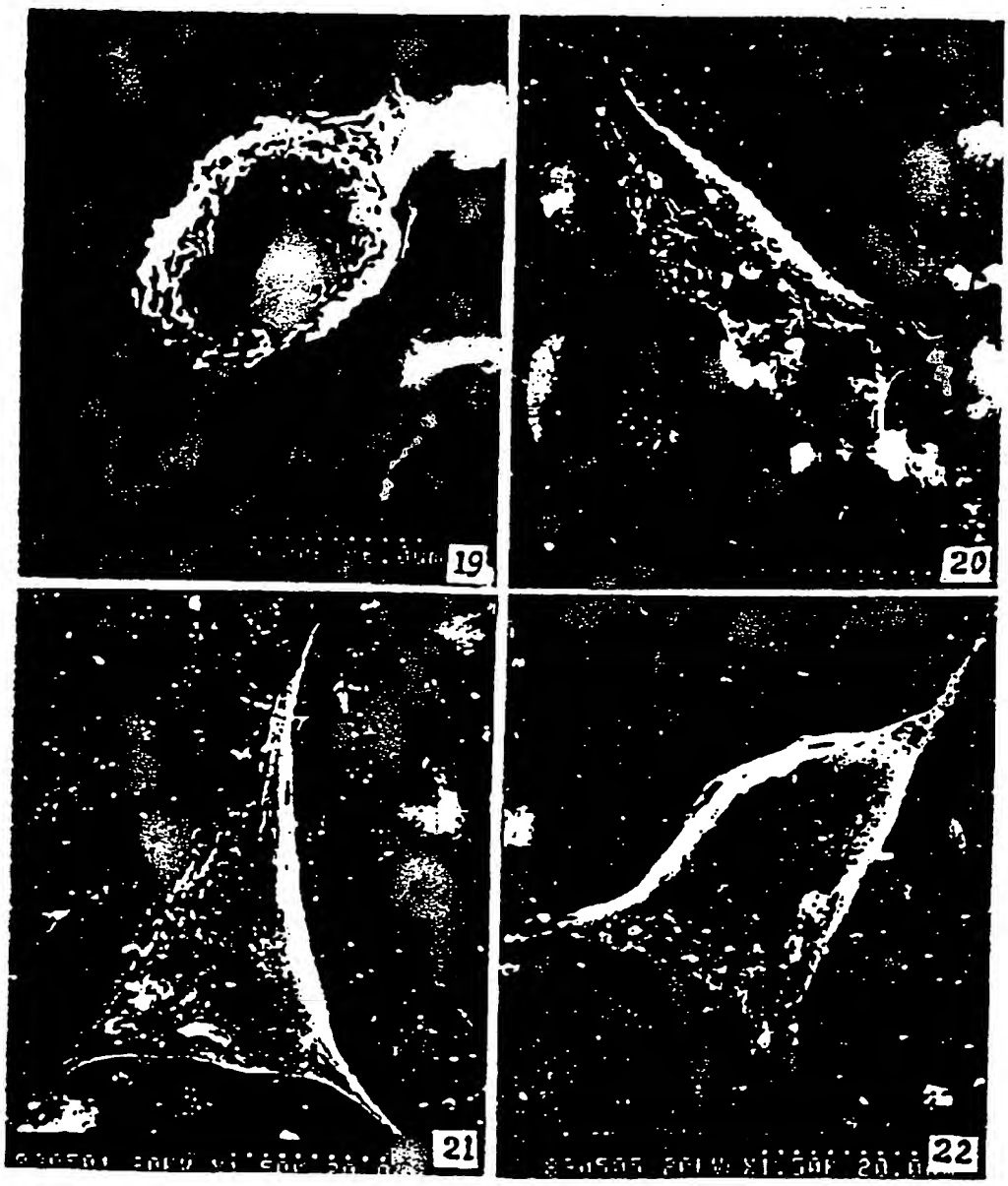

Fig. 6

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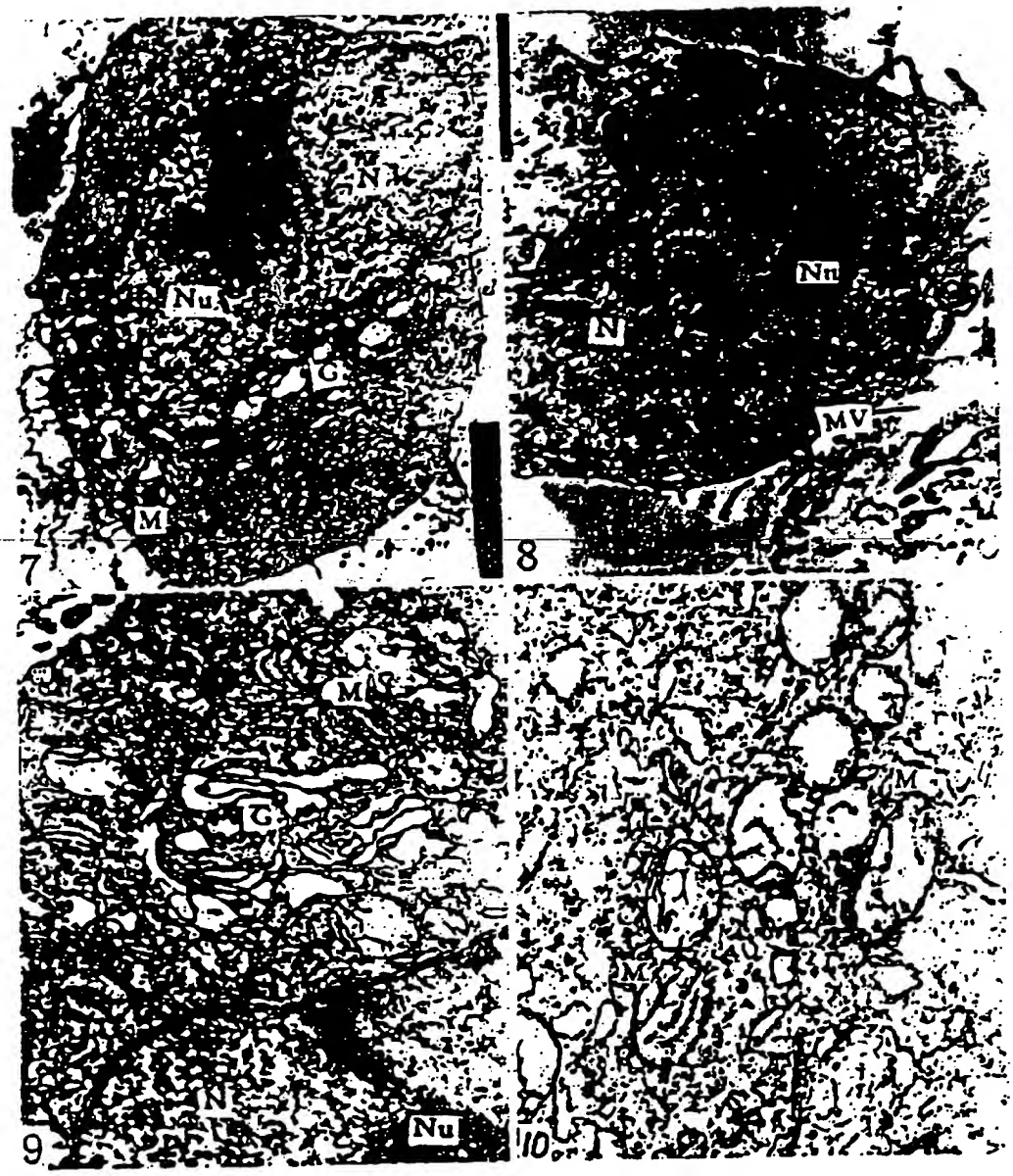

Fig. 7

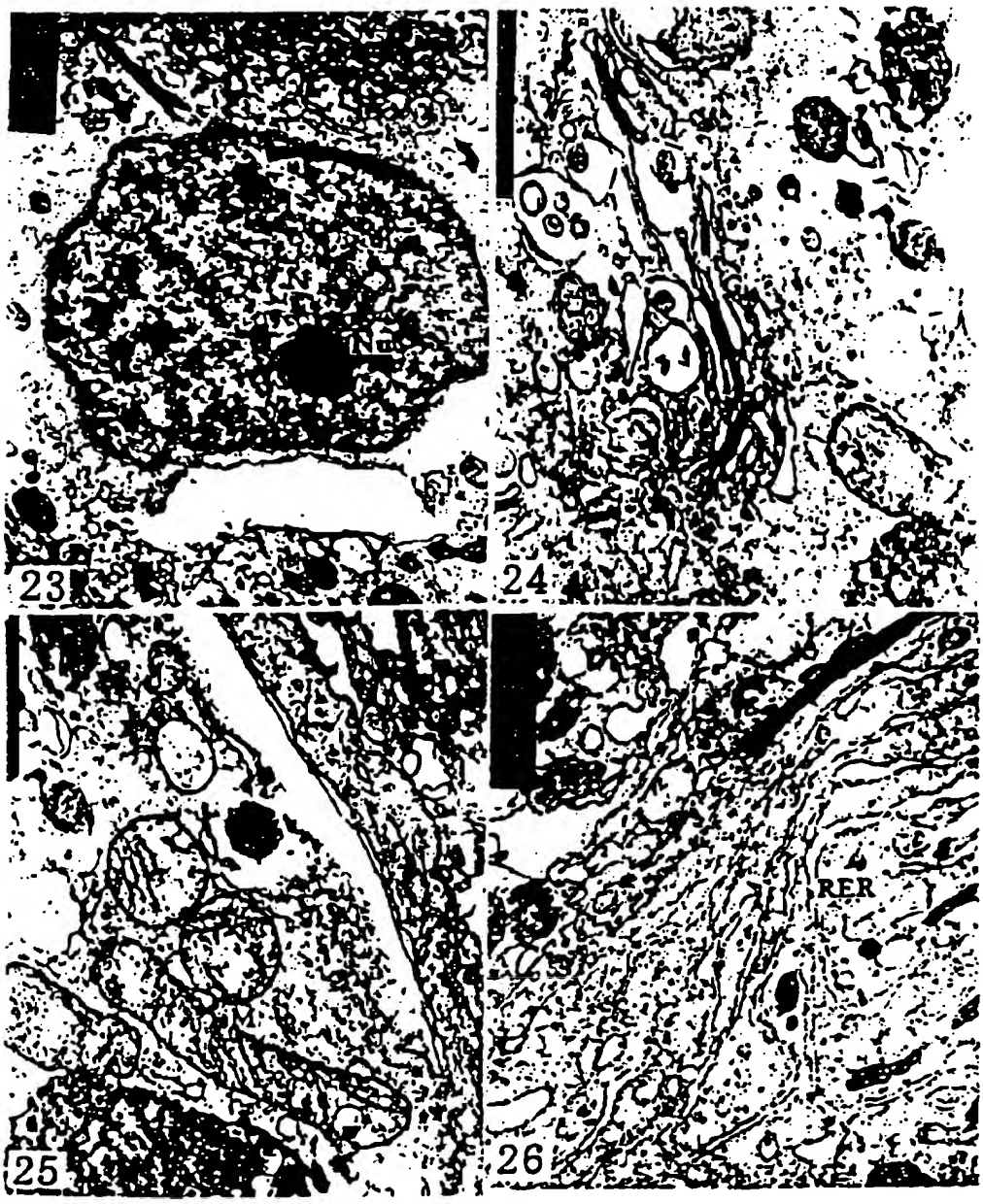

Fig. 8